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doi:10.1016/j.bbabio.2014.05.231

S6.P3

Resolution of ROS

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It remains a challenge to identify the sources and track the quantities and rates of formation of the individual components of reactive oxygen species (ROS) as well as how they are targeted to physiological productive or uncontrolled destructive effects in cells and organelles. We have designed and created manmade maquette proteins dedicated to reveal first principles of protein engineering that govern the individual formation rates and yields of ROS. A series of closely related one or two ferrous heme B maquettes are rapidly exposed to O_2 in a stopped flow spectrometer and analyzed in the millisecond timescale for ferrous heme oxidation and the fate of the oxygen. The ferrous heme (Em8 value −280 mV) can readily singly reduce O_2. We show that a maquette designed to stably bind one or two O_2 as the oxyferrous state releases no detectable ROS on the second timescale of the experiment. A design that disables heme O_2 binding leads to long-range heme to O_2 electron transfer to form superoxide. We follow the millisecond timescale delay as superoxide dismutates to peroxide (second-order) and then on the same timescale the further interaction of superoxide with peroxide to generate a substantial yield of hydroxyl radicals. Alternatively, a di-heme design that promotes water access to the interior allows transient millisecond di-oxyferrous state formation followed by prompt direct internal peroxide formation before its release with little accompanying superoxide or hydroxyl radical states. In analogous maquettes ligating a single two-electron ($n = 2$) flavin, the immediate product is peroxide. We extend this "Fenton Laboratory" to the examination of the molecular events of oxygen with combinations of reduced hemes, flavins and quinones aiming for a deeper understanding of the generation and suppression of ROS and its destructive reactions with a range of biologically and clinically important molecules.

doi:10.1016/j.bbabio.2014.05.232

S6.P4

Mentha aquatica L. extract effects on mitochondrial bioenergetics

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Mentha aquatica (water mint) extracts are regularly used in food flavoring and pharmacology. In the present study, the possible effects of an ethanolic extract from leaves of *M. aquatica* L. on rat liver mitochondria bioenergetics were evaluated.

The plant extract (25 μg·mg protein^{-1}) but not the vehicle, inhibited the mitochondrial oxidative system, as seen by a depression of respiration (state 3, respiratory control ratio (RCR), FCCP-stimulated respiration) and lower generation of the transmembrane electric potential using glutamate + malate and succinate as respiratory substrates. The depressing effects in oxidative phosphorylation can probably be related with the polyphenolic composition of the extract (mainly eriodictyol-7-*O*-rutinoside, luteolin-7-*O*-rutinoside, naringenin-7-*O*-rutinoside, hesperitin-7-*O*-rutinoside and rosmarinic acid) that can interact with membrane and change the inner mitochondrial membrane lipidic moiety. Despite decreasing the RCR, the presence of *M. aquatica* extract did not affect the mitochondrial phosphorylative capacity, as estimated by the ADP/O ratio. No significant increase in inner mitochondrial membrane permeability was observed and induction of mitochondrial permeability transition pore was not altered in the range of concentrations tested (up to 25 μg·mg protein^{-1}) either. For the highest concentrations tested (25 μg·mg protein^{-1} or higher) the inhibition observed on the mitochondrial respiratory chain, as reflected by FCCP-stimulated respiration, revealed that *M. aquatica* ethanolic extract is toxic for mitochondrial bioenergetics. In conclusion, the present study suggests that a high daily consumption of an ethanolic extract of *M. aquatica* leaves should be regarded as hazardous. Acknowledgements: FCOMP-01-0124-FEDER-022696 to CITAB; PEst-OE/AGR/UI0681/2014 to CERNAS, and PEst-C/SAU/LA0001/2013-2014 to the CNC, co-funded by FEDER/Compete and National Budget.

doi:10.1016/j.bbabio.2014.05.233

S6.P5

Does sun-induced mitochondrial mutagenesis and dysfunction interfere with chromosomal DNA repair and contribute to skin cancerogenesis?

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Mitochondria are central in cell physiology as they are the place of energy generation, but also involved in many other essential cellular processes. DNA repair is the main cellular defense line as it prevents mutagenesis in nucleus and mitochondria by the efficient removal of DNA damage. Both, mitochondrial function and DNA repair capacity, decline during ageing. In nucleus, but especially in mitochondria, ageing is associated with an accumulation of DNA mutations. In skin, sun exposure is a factor which dramatically accelerates these processes. The focus of our research is the influence of solar radiation on the function of skin mitochondria. We further research whether mitochondrial dysfunction might causatively contribute to impaired DNA repair and finally cancer. Here we show that UV-exposed skin samples harbored increased levels of the 4,977 bp common deletion and an increased level of mitochondrial DNA (mtDNA)

copies per cell. These effects were not age-dependent. Whereas there were no significant differences in respiration profile between fibroblasts, keratinocytes and melanocytes isolated from young and old donors, irradiation with sun simulating light (SSL) significantly interfered with respiration. Furthermore irradiation also resulted in an up-regulation of mtDNA copy number. To analyze effects on DNA repair we measured nucleotide excision repair (NER) capacity by the ability of the cells to restore an ultraviolet C irradiated reporter plasmid. We chose this pathway as it is the main pathway removing sun-induced DNA damage and thereby preventing skin cancer. Irradiation resulted, however, only in a slight decrease in DNA repair capacity for high SSL doses. To directly analyze the effects of mitochondrial malfunction on NER, we treated human skin fibroblasts with the mitochondrial inhibitors oligomycin, rotenone and antimycin A. Treatments of human skin fibroblasts with mitochondrial inhibitors for 64 h resulted in a metabolic shift from oxidative phosphorylation to glycolysis without affecting cellular replication rate. Surprisingly, two of the inhibitors (oligomycin and antimycin A) induced a 10% increase in NER capacity without changing gene expression of involved genes. We conclude that inhibition of mitochondrial respiration can increase DNA repair capacity via yet unknown mechanisms.

doi:10.1016/j.bbabio.2014.05.234

S6.P6

Pharmacological activation of ischemic tolerance signaling pathways ameliorates gentamycin nephrotoxicity

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The toxic effects of aminoglycoside antibiotics are realized via stimulating intracellular reactive oxygen species (ROS) overproduction. Oxidative damage of mitochondria results in the induction of mitochondrial permeability transition pore (MPTP). Activation of signaling pathways that prevents opening of MPTP is known as preconditioning (PC) phenomenon. PC signaling pathways are converged on glycogen synthase kinase 3β (GSK3β) and results in its inhibition by phosphorylation. The aim of the study was to investigate whether mitochondria-targeted antioxidant SkQR1, δ-opyoid receptor agonist dalargin and GSK3β inhibitor LiCl are able to prevent gentamycin nephrotoxicity. Male outbred rats were treated with gentamycin (i.p. 160 mg/kg/day) for 6 days. Protocol resulted in pronounced nephrotoxicity (3.6-fold increase of serum creatinine), marked mortality and decrement in renal erythropoietin (EPO) content. SkQR1 administration (i.p. 100 nmol/kg) 3 h before each gentamycin injection mitigated renal failure, diminished mortality and normalized EPO level. The single injection of SkQR1 to intact rats was found to induce PC signaling in the kidney: 1.8-fold increase in EPO content and 2-fold increase in p-GSK3β content 3 h and 24 h after SkQR1 injection respectively. The rise in p-GSK3β content was also observed after i.p. injection of δ-opyoid receptor agonist dalargin (i.p. 50 μg/kg) or mood-stabilizing drug LiCl (i.p. 30 mg/kg). Single gentamicyn (i.p. 160 mg/kg) injection caused the 10-fold increase of ROS production in kidney, detected by DCF-DA staining. Dalargin or LiCl mitigated ROS production (15% and 35% decrease respectively) when administrated 3 h before gentamicyn. The long-term gentamicyn treatment resulted in accumulation of carbonylated proteins in the kidney (measured by OxyBlot) and renal function impairment. Pretreatment with dalargin or LiCl before each gentamycin injection resulted in 50% and 35% decrement of carbonylated proteins signal intensity and in reduced kidney injury. We conclude that mitochondria-targeted antioxidant SkQR1 effectively prevented nephrotoxicity of gentamycin. Partially this protective effect could be referred to induction of PC signaling. Moreover, different compounds that inhibit GSK3β thus protecting mitochondria, such as LiCl and dalargin, may serve as promising agents for preventing negative consequences of aminoglycoside therapy. The work was supported by RFBR grants 14-04-00300 and 14-04-00542.

doi:10.1016/j.bbabio.2014.05.235

S6.P7

Delta pH or membrane potential: The role of components of proton motive force in the production of reactive oxygen species of mitochondria

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Mitochondrial production of reactive oxygen species (ROS) has a major role in the generation of oxidative stress in cells. A number of pathological conditions are associated with an increased ROS production. There are controversies considering both the quantitative and qualitative aspects of mitochondrial ROS production. One of these questions is which component of the proton motive force would determine the intensity of mitochondrial ROS production. It is known that those mitochondria which are the most hyperpolarized and have the highest ΔpH produce a very high rate of ROS. In the present study we tried to dissect these two components of the proton motive force (ΔΨm and ΔpH) and determine which of them has a major role in ROS formation. Guinea pig brain mitochondria were used throughout the study and mitochondrial respiration was supported with substrates which were able to initiate the reverse flow of electrons in the respiratory chain. ROS (hydrogen peroxide) formation was measured by the Amplex Red fluorescent dye, mitochondrial membrane potential with safranin fluorescence, mitochondrial matrix pH was determined by BCECF fluorescence. In the presence of an uncoupler ΔΨm was dissipated and ΔpH equilibrated with a corresponding decrease in ROS production. In order to dissect the ΔΨm from the ΔpH, ionophores (valinomycin and nigericin) were used. Using K^+ ionophore valinomycin at low [K^+] in the absence of inorganic phosphate, membrane potential was decreased, as a consequence ΔpH increased and matrix pH rose with a corresponding decrease of H_2O_2 production. Upon addition of nigericin, a K^+/H^+ exchanger, mitochondrial pH gradient was decreased with a concomitant increase in ΔΨm, leaving the proton motive force unchanged; under these conditions, only minor changes were detected in the ROS production. Since both ionophores altered the intramitochondrial pH we considered matrix pH as a possible independent variable playing a role in ROS formation. Stepwise elevation of the pH of the buffer solution elevated succinate supported mitochondrial ROS production. Our studies with ionophores show that out of the two components of proton motive force, ΔΨm seems to play a greater role in ROS formation compared to ΔpH. Our results also show that matrix pH may also be a